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TFAR19, a Novel Apoptosis-Related Gene Cloned from Human Leukemia Cell Line TF-1, Could Enhance Apoptosis of Some Tumor Cells Induced by Growth Factor Withdrawal

Hongtao Liu, Yugang Wang, Yingmei Zhang, Quansheng Song, Chunhui Di, Guanghui Chen, Jian Tang, and Dalong Ma
Laboratory of Medical Immunology, Beijing Medical University, Beijing 100083, People's Republic of China

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Using the cDNA-representative differences analysis (cDNA-RDA) approach, we identified a novel gene, *TFAR19* (TF-1 cell apoptosis related gene-19), from TF-1 cells undergoing apoptosis. The human *TFAR19* encodes a protein which shares significant homology to the corresponding proteins of species ranging from yeast to mice. *TFAR19* exhibits a ubiquitous expression pattern and its expression is upregulated in the tumor cells undergoing apoptosis. Overexpression of TFAR19 in tumor cells enhances apoptosis triggered by growth factor or serum deprivation. We propose that TFAR19 may play a general role in the apoptotic process.

Apoptosis or programmed cell death (PCD) is a genetically controlled program of cellular self-destruction, which is of central importance to the development and homeostasis of virtually all animals (1). It is a highly regulated process involving a large number of genes that are conserved in all metazoans. It can be induced by a variety of stimuli, including deprivation of growth factors, signaling via certain surface receptors, treatment with DNA-damaging agents and inhibitors of macromolecular synthesis (1, 2). A variety of genes have been found that take part in the process of apoptosis, such as the TNF/FasL family, the Bcl-2 family, the ICE/CED-3 family, oncogenes, tumor suppresser genes and the genes encode heat shock proteins (3). The TF-1 cell line is a human premyeloid cell line established from a patient with erythroleukemia. Its survival and proliferation are dependent on IL-3, GM-CSF, IL-13, EPO, and other cytokines (4). Flow cytometric analysis showed that 8 h after IL-3 deprivation approximately 70% of cells enter apoptosis (5). Cyclohexamide, an inhibitor of protein synthesis, can block the apoptosis of the cell line which indicates that the active nature of apoptosis in TF-1 cells may be dependent on protein synthesis in the dying process and newly-transcripted genes may be involved in the regulation (6). To further study the mechanism of apoptosis in the TF-1 cells and find the novel genes with important functions in this process, we applied the cDNA-RDA (cDNA-representative differences analysis) method to study the differentially expressed genes involved in the process of apoptosis of TF-1 cells. We found a novel gene highly expressed in TF-1 cells 8 h after deprivation of GM-CSF in culture medium. Based on the EST sequence, we cloned the full length of the novel gene, *TFAR19* (TF-1 cell apoptosis related gene-19), by the RACE method and further studied its functions in the apoptosis of TF-1 and other tumor cells.

MATERIALS AND METHODS

Cell lines and cell culture. The TF-1 cell line (6) was kindly provided by Professor Ding Xishen of the National Institute for Control of Pharmaceutical and Biological Products, PR China and maintained in RPMI 1640 medium supplemented with penicillin/streptomycin, 10% fetal bovine serum, and 80–100 units/ml human recombinant GM-CSF. MGC-803 cells, a human stomach tumor cell line provided by Professor Dong Zhiwei of the Chinese Academy of Medical Science (7), and Hela cells were maintained in the same medium except human recombinant GM-CSF was not added. The transfectant lines from MGC-803 and Hela cells were maintained in standard medium supplemented with 1 mg/ml Geneticin (G-418, Gibco).

RDA. The RDA procedure was performed as described using *Dpn*II restriction endonuclease (8–10). TF-1 cells, which underwent withdrawal of cytokine for 8 h, were used as the tester while normally cultured TF-1 cells as the driver. Briefly, approximate 2 μg tester (without GM-CSF) and 2 μg driver (with GM-CSF) cDNAs were digested with DpnII and ligated to adapters. The amplicons were amplified by polymerase chain reaction (PCR) amplification. After three rounds of competitive hybridization, the final PCR products were again digested with DpnII to remove adapters, and then the resultant mixture was cloned into pGEM3Zf previously digested with BamHI and dephosphorylated with calf intestinal phosphatase. White colonies from transformed IQ bacteria were screened by colony

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PCR. The positive clones were sequenced and the similarity search was conducted through GenBank.

Slot blots and Northern blots. In slot blots, a total of 10 μg tester and driver total RNAs were transferred onto a Gene Screen Plus [Hybridization Transfer Membrane (DuPont, Boston, MA)]. When conducting Northern blots, about 10 μg of total RNA were run through gels containing formaldehyde and formamide, and then transferred onto nylon filters by capillary method transfer. The labeling of probe and hybridization was performed according to the protocol of random primer fluorescent labeling kit with antifluorescein–HRP (DuPont NEN, Boston, MA) provided by the company.

Rapid amplification of cDNA ends (RACE) procedure and sequencing. The 5′ and 3′ RACE reactions were performed using the Marathon cDNA Amplification Kit following the manufacturer's instructions (Clontech) cDNA from TF-1 cells harvested at 8 h after the deprivation of GM-CSF in culture medium was used in the reaction. TFAR19 cDNA sequence was obtained by using ALFTM DNA Sequencer (Pharmacia Biotech, Uppsala, Sweden). Sequencing reactions were done according to the instruction of the Standard Annealing of Primer to Double-Strand Template in the Auto Read sequencing kit.

Plasmid construction. The entire *TFAR19* coding region was amplified by PCR using upstream and downstream primers, which introduce a XhoI site at 3′ of the *TFAR19* Coding region. The PCR products were blunted by Klenow enzyme and cut by XhoI. After recovering from gels, the fragment was ligated into the pMTY4 plasmid at *Stu*I and *Xho*I sites and the recombinant plasmid was designated as pMTY4TFAR19 and fully sequenced. The prokaryotic expression vector pMTY4 was constructed in our laboratory previously by the insertion of the multiple cloning sites of the pCI Vector (Promega) and a thrombin linker into the *E. coli* expression plasmid pEX31 (11). Amplification was performed in a thermocycler programmed for PCR and the 386-bp PCR product containing the coding region of TFAR19 gene was than cloned into the pGM-T Easy vector (Promega) for lange amplification. To construct the eukaryotic expression vector, the fragment containing the ORF of TFAR19 was released from the recombinant plasmid by cutting with EcoRI and subcloned into the *Eco*RI site of vector pcDI, which was constructed by substituting the *Bgl*II–*Kpn*I fragment of pcDNA3 (Invitrogen) (12) with the *Bgl*II–*Kpn*I fragment of pCI. The sense pcDITFAR19 (+) and antisense pcDITFAR19 (−) expression vectors were selected by restriction enzyme analysis.

Preparation of polyclonal antibody to TFAR19 and Western blotting. The *E. coli* pop2136 harboring the prokaryotic expression vector pMTY4TFAR19 was heated to induce the expression of the MS2-TFAR19 fusion protein. After denaturing, renaturing and cleavage with thrombin, TFAR19 was purified by ion exchange chromatography with DEAE Sepharose Fast Flow (Pharmacia). The polyclonal antibodies against *TFAR19* was prepared by immunizing BALB/c mice with purified recombinant protein. Cellular Proteins from TF-1 cells cultured in GM-CSF-containing medium and from TF-1 cells harvested at 8 h after deprivation of GM-CSF from culture medium were purified by Trizol reagent (GIBCO). Protein concentrations were measured using the DC protein assay kit (Bio-Rad). Lysates containing equal amounts of proteins were subjected to SDS–polyacrylamide gel electrophoresis (SDS–PAGE; 15%), followed by transfer to a Bio Trace PVDF membrane (Gelman). The membrane was preblocked in PBS containing 2% nonfat milk (blocking buffer) and for 1 h and then treated with the mouse polyclonal antibodies against TFAR19 at a 1:50 dilution in blocking buffer for 1 h. After incubation with the primary antibodies, the membrane was washed three times (5 min each) with PBS and then incubated for 1 h with alkaline phosphatase (AP)-conjugated secondary antimouse IgG (Promega) that had been diluted 1:1000 in blocking buffer. The membrane was again washed three times with PBS and immunoreactive proteins were visualized by adding BCIP and NBT substrates (Promega).

Transfection and transfectants. The cells were transfected with Lipofectin Reagent according to the procedure provided by the manufacturer. For each transfection, 10 μl Lipofectin Reagent and 1 μg plasmid DNA were used. For stable transfection, a final concentration of 0.4 mg/ml of G-418 was added 2 days after transfection. The cells were cultured with the concentration of G-418 gradually increased to 1 mg/ml among 15–20 days. Slot RNA blot and slot genomic DNA blot were used to identify the positive cells.

Analysis of effects of TFAR19 on cell death. The apoptosis of TF-1 cells transfected with the eukaryotic expression plasmids was measured in the following condition: with full dosage of GM-CSF, with semi-dosage of GM-CSF and with deprivation of GM-CSF in the culture medium for 8 h. Apoptosis was detected using the ApoAlert Annexin V apoptosis Kit (Clontech) according to the user manual. Mean Fluorescein Intensity was detected by flow cytometry (Becton–Dickinson) at the wave length of 488 nm. The percentage of apoptotic cells of the MGC-803 and Hela cell stable transfectants was determined 48 and 96 h after withdrawal of serum from culture medium by propidium iodide staining and FACS (13).

Other procedures. The expression levels of *TFAR19* mRNA in different tissues and developmental stages were determined using The Human RNA Master Blot (Clontech) according to the user manual provided by the manufacturer.

RESULT

Identification of human TFAR19. In the present study, we used the TF-1 cell line in our model system to identify genes which are involved in the apoptotic process of hematopoietic cells triggered by growth factor withdrawal. Consistent with the published report (5), we found that 70% of TF-1 cells entered apoptosis 8 h after GM-CSF deprivation (data not shown). Therefore, we focused our effort on the identification of the genes that are induced by the 8-h GM-CSF deprivation. Based on the BLAST search through NCBI Database, we found that 6 of 20 clones represent novel genes. The fact that we identified genes, such as thioredoxin, which have been shown to be upregulated in apoptotic cells, indicates that our approach to identifying differentially regulated genes was successful. Because of the dramatic upregulation at the message level and relatively small message size, the Clone 19 (referred to as *TFAR19* was chosen for further analysis). Figure 1 showed that *TFAR19* was expressed higher in TF-1 cells cultured in GM-CSF-free medium than in TF-1 cells cultured with GM-CSF. Results from Northern blot indicated that the size of TFAR19 mRNA is approximately 600 bp. The initially obtained cDNA fragment of *TFAR19*, however, is about 290 bp, and therefore, we performed a 5′ RACE reaction using the total RNA isolated from TF-1 cells to clone the 5′ untranslated region and rest of the coding region. Multiple clones derived from the 5′RACE were sequenced to confirm the sequence authenticity. Sequence analysis revealed that TFAR19 encodes a protein of 125 aa, with an ATG initiation codon at nt 25–27. Its surrounding sequence (GCCATGG) complied with the Kozak's con-

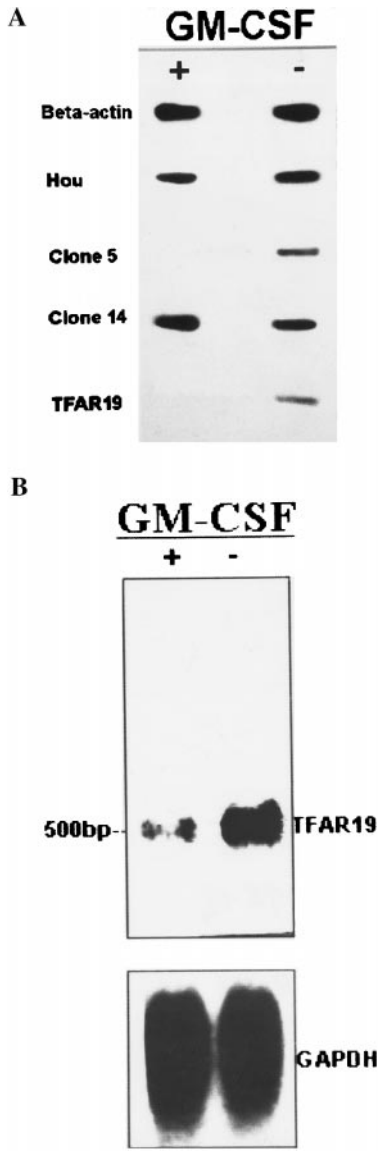

FIG. 1. Detection of *TFAR19* mRNA expression in TF-1 cells by slot blot and Northern blot. (A) the result of slot blot and (B) the result of Northern blot; Left is RNA from TF-1 cells undergoing apoptosis, right is RNA from normal-cultured TF-1 cells.

sensus sequence for initiation of translation (14) (Fig. 2A). Prosite search indicated that TFAR19 contain one cAMP- and cGMP-dependent protein kinase phosphorylation site (aa 97–100), multiple protein kinase C phosphorylation sites (aa 51–53, aa 84–86, aa 103–105, aa 108–110), and a casein kinase II phosphorylation site (aa 119–122). Using a homology search, we found that the human TFAR19 protein is homologous to gene products from species ranging from yeast to mice. This indicates that TFAR19 protein is a well conserved protein (Fig. 2B). By the assemble of several mouse EST sequences, we obtained a gene which encodes a protein with 96% homology to human TFAR19. This suggests that it is mouse homologs of human TFAR19 (this was confirmed later on by cloning the full length cDNA of the mouse TFAR19 gene from a mouse heart cDNA library). To our surprise, we also found rice EST sequence (AA675744 and D39240) may encode a protein homologue to *TFAR19*. The predicted amino acid sequence does not contain any signal sequence, transmembrane domain, ER retention site, and mitochondrial targeting sequence, based on analysis done on the PSORT WWW Server (http://psort.nibb.ac.jp). However, using Reinhardt's method to predict subcellular localization of protein (15), TFAR 19 was predicted to localize in the nucleus.

Expression of TFAR19. TFAR19 mRNA is not hemapoietic restricted and it expresses in all of the fifty human tissues detected by the Master blot (Fig. 3), especially in the heart, testis, kidney, pituitary gland, adrenal gland and placenta. Remarkably, the expression of *TFAR19* mRNA in fetal tissues is significantly lower than that in adult tissues. The ubiquitous expression pattern of *TFAR19* detected by dot blot (Fig. 3) is consistent with the finding that EST sequences corresponding to human and mouse *TFAR19* were also expressed in a wide variety of tissue sources.

The protein of TFAR19 expresses highly in apoptotic TF-1 cells. TFAR19 was expressed in E. coli and purified by DEAE column chromatography. The purity of the purified protein was higher than 90% as assayed by ImageMaster DTS (Pharmacia LKB, data not shown). The polyclonal antisera against human TFAR19 were generated by immunizing Balb/c mice with the purified recombinant TFAR19 protein and used in Western blot analysis. A unique band at about 14 kDa was detected in cells transferred with TFAR19 cDNA. No band was evident in the blot of the same samples using a preimmune mouse serum. Using growth factor deprivation we next examined whether or not TFAR19 protein was induced in TF-1 cells. We found that in TF-1 cells subjected to GM-CSF deprivation for 8 h TFAR19 protein levels were about three- to fourfold that of levels in normally cultured TF-1 cells. This parallels the induction of *TFAR19* mRNA (Fig. 4).

Overexpression of TFAR19 in tumor cells enhances apoptosis. To test if TFAR19 plays a role in the apoptosis, we transfected TFAR19 expression constructs into TF-1, MGC-803, and Hela cells and examined the effect of over-expression of TFAR19 in those cells on the apoptotic process triggered by either GM-CSF or serum deprivation. Compared to the mock-transfected cultures, there was a increase by 200% of apoptotic cells in cultures of TF-1 cells transfected with TFAR19 (Fig. 5A). We also noted that TFAR19 itself could not induce or trigger apoptosis of TF-1 cells in the presence of GM-CSF, whether at full or semi-full dosage (data not shown). The growth curves of stable transfectants of tumor cells showed that MGC-803 cells transfected with the TFAR19 sense gene grew much slower than

A

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CTG CTC CAG CGC TGA CGC CGA GCC ATG GCG GAC GAG GAG CTT GAG
                            MET Ala Asp Glu Glu Leu Glu

GCG CTG AGG AGA CAG AGG CTG GCC GAG CTG CAG GCC AAA CAC GGG 90
Ala Leu Arg Arg Gln Arg Leu Ala Glu Leu Gln Ala Lys His Gly

GAT CCT GGT GAT GCG GCC CAA CAG GAA GCA AAG CAC AGG GAA GCA
Asp Pro Gly Asp Ala Ala Gln Gln Glu Ala Lys His Arg Glu Ala

GAA ATG AGA AAC AGT ATC TTA GCC CAA GTT CTG GAT CAG TCG GCC 180
Glu MET Arg Asn Ser Ile Leu Ala Gln Val Leu Asp Gln Ser Ala

CGG GCC AGG TTA AGT AAC TTA GCA CTT GTA AAG CCT GAA AAA ACT
Arg Ala Arg Leu Ser Asn Leu Ala Leu Val Lys Pro Glu Lys Thr

AAA GCA GTA GAG AAT TAC CTT ATA CAG ATG GCA AGA TAT GGA CAA 270
Lys Ala Val Glu Asn Tyr Leu Ile Gln MET Ala Arg Tyr Gly Gln

CTA AGT GAG AAG GTA TCA GAA CAA GGT TTA ATA GAA ATC CTT AAA
Leu Ser Glu Lys Val Ser Glu Gln Gly Leu Ile Glu Ile Leu Lys

AAA GTA AGC CAA CAA ACA GAA AAG ACA ACA ACA GTG AAA TTC AAC 360
Lys Val Ser Gln Gln Thr Glu Lys Thr Thr Thr Val Lys Phe Asn

AGA AGA AAA GTA ATG GAC TCT GAT GAA GAT GAC GAT TAT TGA ACT
Arg Arg Lys Val MET Asp Ser Asp Glu Asp Asp Asp Tyr END

ACA AGT GCT CAC AGA CTA GAA CTT AAC GGA ACA AGT CTA GGA CAG 450
AAG TTA AGA TCT GAT TAT TTA CTT TGT TTA TTG TCT ATA TGC CTT
TTA AAA AAA TAA ACT TGT TAT GCA AAA AAA AAA AAA AAA AAA AAA 540
AAA AAA AAA AAA AAA AAA A
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B

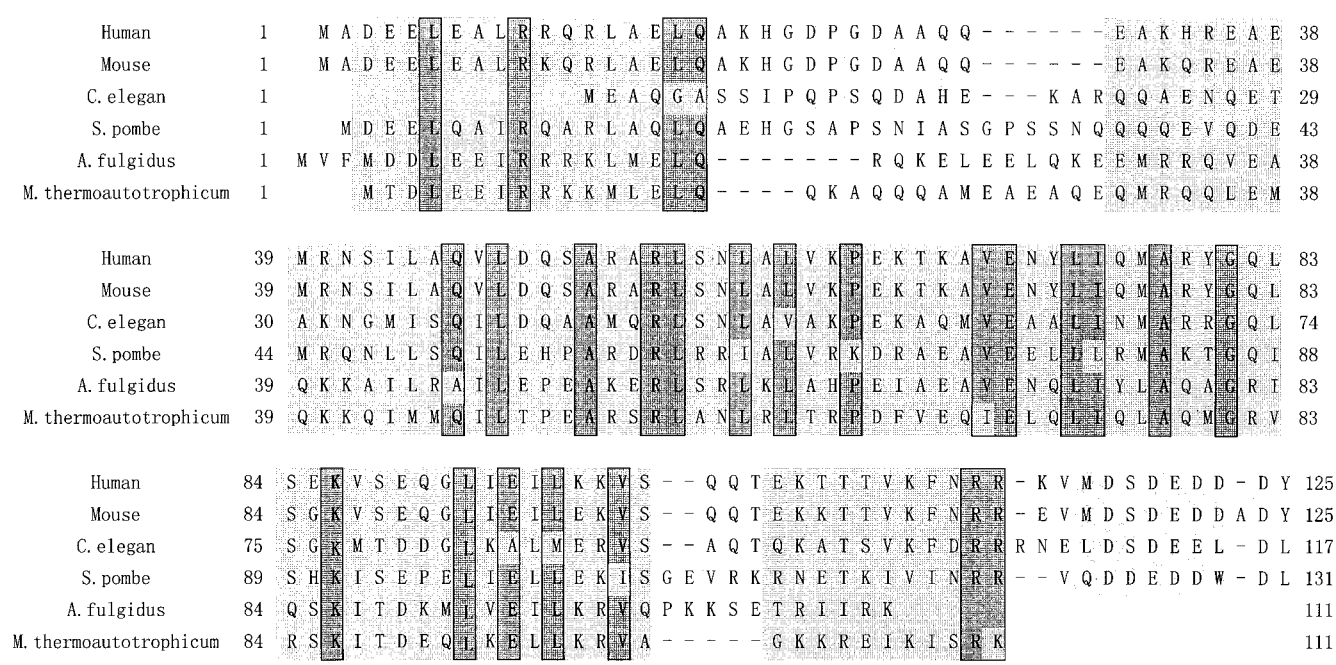

FIG. 2. Nucleotide and Predicted Amino Acid Sequence of the *TFAR19* cDNA and sequence alignment of TFAR19 with the protein from other species. (A) The bold and underlined Ser is the possible location of a cAMP-and cGMP-dependent protein kinase phosphorylation site. (B) Alignment was done by the Needleman–Wunsch algorithm method. Dark gray background and solid bar shows identity with at lease five aligned residues in different species. The gray region represents the sequence conserved region among different species.

non-transfected and control plasmid-transferred cells (Fig. 5B). However, the phenomenon was not as apparent in Hela cells (data not show). When transfectants of MGC-803 and Hela cells were deprived of serum in the culture medium, the cells with the *TFAR19* sense gene entered apoptosis faster than those with the control

A

	1	2	3	4	5	6	7	8
A	whole brain	amygdala	Caudate nucleus	cerebellum	cerebral cortex	frontal lobe	hippocampus	medulla oblongata
B	occipital lobe	putamen	Substantia nigra	temporal lobe	thalamus	subthalamic nucleus	spinal cord	
C	heart	aorta	Skeletal muscle	colon	bladder	uterus	prostate	stomach
D	testis	ovary	Pancreas	pituitary gland	adrenal gland	thyroid gland	salivary gland	mammary gland
E	kidney	liver	Small intestine	spleen	thymus	peripheral leukocyte	lymph node	bone marrow
F	appendix	lung	Trachea	placenta				
G	fetal brain	fetal heart	Fetal kidney	fetal liver	fetal spleen	fetal thymus	fetal lung	

B

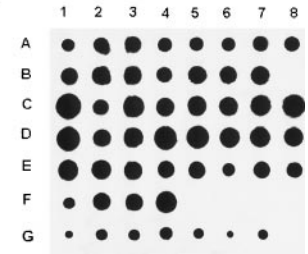

FIG. 3. Expression of *TFAR19* mRNA in different tissues. (A) the position of different tissues on the hybridization membrane, (B) the result of dot hybridization.

plasmid. In addition, the cells with the *TFAR19* antisense gene entered apoptosis slower than those with the control plasmid (Fig. 5C). This suggests that TFAR19 may play a role in the process of cell growth and apoptosis of tumor cells. We confirmed the exogenous expression of *TFAR19* in the transfectants by Western blotting (data not shown). These results indicate that TFAR19 may play a general role in enhancing the apoptotic process.

DISCUSSION

The mechanisms of apoptosis of TF-1 cells and other cytokine-dependent hemapoietic cells have been extensively studied (5, 6, 16–19). Using IL-3-dependent myeloid cells as models of apoptosis, some novel genes that are transcriptionally activated during programmed cell death or encode transcription factors involved in triggering apoptosis have been isolated (20, 21). The initiation of apoptosis requires gene transcription, protein synthesis and phosphorylation of proteins. The fact that cell death can be repressed by inhibitors of RNA or protein synthesis suggests that apoptosis of TF-1 cells under the condition of cytokine deprivation is controlled at the transcriptional level. In our study, a novel human cDNA, *TFAR19,* was cloned by the cDNA-RDA method from TF-1 cells which underwent withdrawal of cytokine from culture medium for 8 h. TFAR19 expressed highly in the process of apoptosis of TF-1 cells as detected by both Northern blot and Western blot. Part of its sequence is homologous proteins functioning in the nucleus. This shows that TFAR19 might play a role in the regulation of gene transcription in the nucleus. By searching the GenBank, it was also found that the amino acid sequence of TFAR19 is well conserved in several different species, including mice, Caenorhabditis elegans, Schizosaccharomyces pombe, *Archaeoglobus fulgidus,* and *Methanobacterium thermoautotrophicum* (Fig. 2B). The functions of these proteins are also unknown. The conservation of the gene implies that it may have a very important function, since it remains stable during the evolution of species.

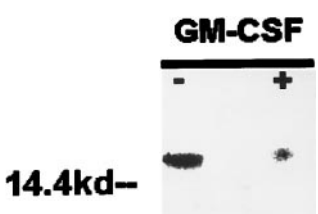

FIG. 4. The Western Blot shows that the protein of TFAR19 expressed higher in apoptotic TF-1 cells than in normally cultured TF-1 cells.

There are four possible phosphorylation sites for protein kinase C (PKC) and one possible site for cAMP- and cGMP-dependent protein kinase in TFAR19. This suggests that TFAR19 may be a substrate of some phosphorylation kinases and its phosphorylation may regulate its function. The mechanism by which TFAR19 enters the nucleus and is activated remains to be established.

The abundance of *TFAR19* mRNA in TF-1 cells was increased in response to GM-CSF deprivation, thus we know that the function of TFAR19 in apoptosis is controlled at the transcriptional level. The functional study showed that in the presence of cytokine, overexpression of TFAR19 neither altered normal cell viability nor induced the apoptosis of TF-1. However, overexpression of TFAR19 accelerated the apoptosis of TF-1 cells induced by the withdrawal of cytokine from the culture medium (Fig. 5A). Moreover, overexpressed TFAR19 accelerated apoptosis of some tumor cells (MGC-803, Hela) following serum deprivation (Fig. 5C). These results indicate that TFAR19 could enhance the effects of other apoptotic inducers.

Preliminary studies also showed that TFAR19 alone could inhibit the growth of some tumor cells. Further research will determine whether there is any relationship between the growth inhibition and apoptosis promotion of TFAR19. It should be pointed out that TFAR19 has different effects on different tumor cells.

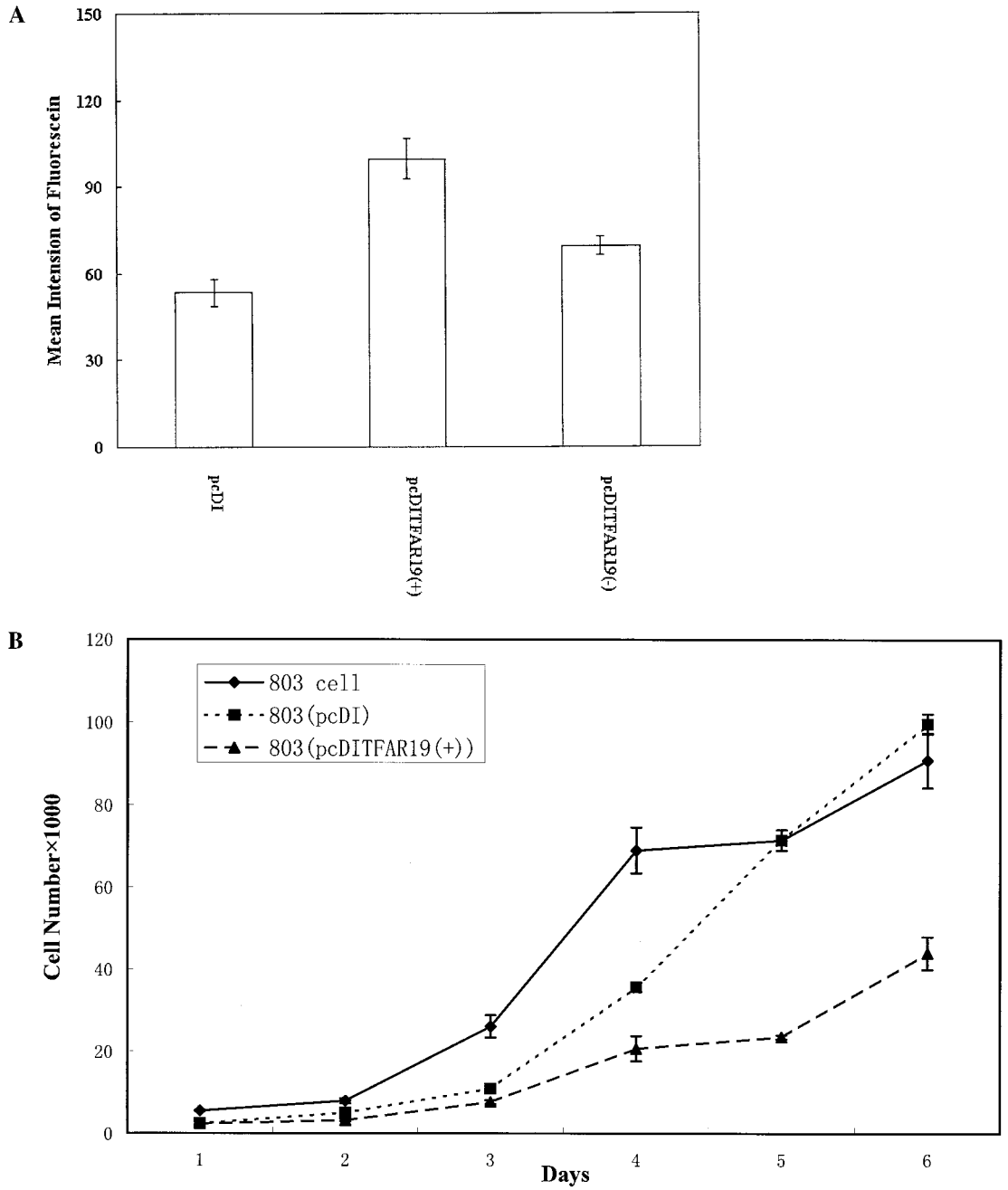

FIG. 5. TFAR19 could enhance the apoptosis of TF-1, MGC-803 cells and Hela cells to enter apoptosis when the cytokine or serum was deprived from the culture medium. (A) pcDITFAR19(+) and pcDITFAR19(−) were transiently transfected into TF-1 cells followed by withdrawal of cytokine from the culture medium. (B) When stably transfected into MGC-803 cell, TFAR19 slowed down the growth of the tumor cells *in vitro*. (C) TFAR19 promoted the apoptosis of MGC-803 and Hela cells induced by growth factor withdrawal. The percentage of apoptotic cells after deprivation of serum for 48 and 96 h was detected by FACS.

For example, overexpression of TFAR19 inhibited the growth of MGC-803 cells *in vitro* (Fig. 5B), but had no influence on that of Hela cells. On the other hands, deprivation of growth factor from culture medium and overexpression of TFAR19 accelerated the apoptosis of both MGC-803 cells and Hela cells (Fig. 5C).

In general, there is a balance between the effector and suppressor of apoptosis in mammalian cells. Under the effect of some apoptosis enhancers, the balance is broken and the cell is destined to die. Several protein families whose members are products of constitutively expressed genes that either stimulate or inhibit apoptosis have been identified (25, 26). Among them, the Bcl-2 family is most important in the apoptosis of TF-1 cells and other IL-3-dependent cells. Bcl-2 confers a death sparing effect to certain hematopoietic cell lines

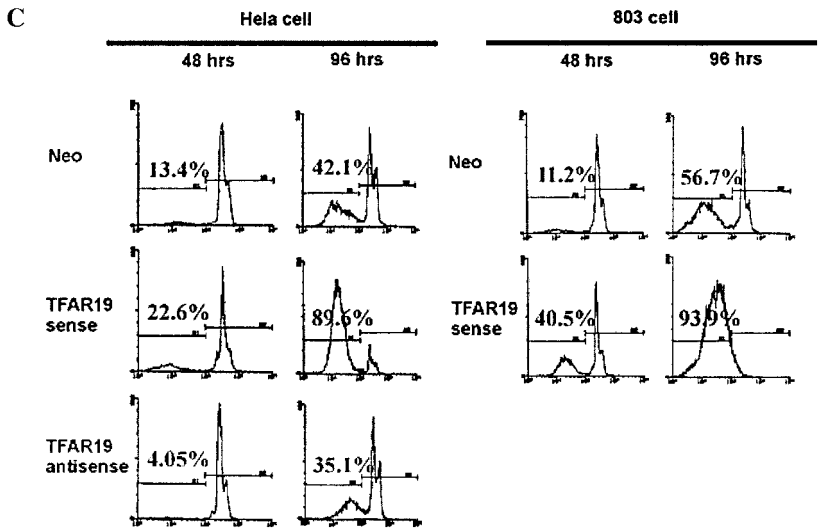

FIG. 5—*Continued*

following growth factor withdrawal (5, 16, 27, 28). On the other hand, overexpression of members of Bcl-2 family, such as Bax (29), Bad (30), harahiri (31), and Bim (32), in IL-3-dependent cell lines could accelerate the apoptosis induced by withdrawal of cytokine. As transcription factors, the presence and overexpression of p53 and c-myc also play an important part in the above apoptosis course (18, 33–37). Among them, Bax and p53 could also inhibit the growth of tumor cells. Although TFAR19 exhibits no homology to these known apoptosis genes, it shares many common characteristics with the above molecules and may be a member of a novel gene family involved in the regulation of apoptosis. The relationship of TFAR19 with the above molecules and the interaction among them should be further studied before we can understand more about the function of TFAR19 in apoptosis.

Master blot analysis of mRNA from a survey of tissues indicated that TFAR19 was not hemapoietic restricted but was widely expressed in a variety of tissues (Fig. 3). The expression of TFAR19 in adult heart, testis, kidney, pituitary gland, adrenal gland and placenta was higher than that in other tissues. This indicated that TFAR19 is not only conserved in evolution but also has an important role in regulating the function of many tissues. It was interesting that the expression of TFAR19 mRNA in fetal tissues was significantly lower than that in adult tissues (Fig. 3). This implies that TFAR19 has more important functions in adult than in fetuses. The function of TFAR19 in other cell and tissue types is presently unknown. The fact that we could not obtain stable-transfected MGC-803 cells with antisense TFAR19 in our experiments indicates that TFAR19 is very important for the survival of MGC-830 cells.

In summary, TFAR19 is a novel human gene conserved in evolution and possessing the ability to accelerate apoptosis under some conditions. It may function in the nucleus and play an important role in the signal transduction to regulate the survival, proliferation, differentiation and apoptosis of cells. At the same time, several questions arose during our study and need to be answered by future research. Which proteins does TFAR19 interact with? How does TFAR19 play a role in growth inhibition and apoptosis promotion? Does TFAR19 function in the fetal stage? Are there any relationship between the expression of TFAR19 and tumorigenesis? It will be very interesting and worthy to further study these aspects.

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